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RESEARCH ARTICLE

Impact of alpha-amylase enzyme on the Rheological and Microstructural properties of the different types of rice flour doughs and bread

Vikendra Dabash*, Iva Buresova

Tomas Bata University in Zlín, Faculty of Technology, Department of Food Technology, nám. T.G. Masaryka 5555, 76001, Zlin, Czech Republic

ABSTRACT

The purpose of this study is to discuss the significant role of the α-amylase enzyme in the production and rheological characteristics of gluten-free rice dough and baked bread. Small deformation and large deformation methods were used to evaluate the effect of the α-amylase enzyme addition on the dough and bread rheological characteristics. The baking properties of bread were evaluated by specific volume, baking loss, and Texture profile analysis parameters. The effect of the added enzyme on the dough microstructure was also evaluated. Small deformation study of doughs showed the highest significant changes after enzyme addition in 0.5S and extra-fine flour doughs. The dynamic rheological study of the flours doughs during the heating period showed significant changes in doughs properties as a function of α-amylase. After enzyme addition, a significant difference in crumb hardness was observed in the fine, semi-coarse, and extra-fine bread. After α-amylase addition in black rice, 0.5S and extra-fine doughs G′, G″ and values were decreased. The research explains how the alpha-amylase enzyme effect the rheological characteristics of gluten-free dough and bread. Rheological analysis of doughs made from flour during the heating period indicated that the properties of the doughs were significantly affected by the presence of α-amylase.

Keywords: α-amylase; Bread; Rheology; Gluten-Free; Rice flour

INTRODUCTION

The specific characteristics of bakery products make flour an essential component of bread production. Flour contains a variety of nutrients, including protein, starch, other carbohydrates, fiber, lipids, water, as well as small amounts of vitamins, minerals, and enzymes (Khatkar, Bell, and Schofield, 1995). Some gluten-free (GF) products on the market now are of poor quality, exhibit reduced mouthfeel, or lack a great deal of flavor, implying that the baking of gluten-free products is a major issue (Ngemakwe, Le Roes-Hill, and Jideani, 2015). A gluten-free (GF) product is defined by the Codex Alimentarius as any food made with naturally gluten-free ingredients that contain less than 20 ppm of gluten (Codex Alimentarius Commission 2015). GF products have become an essential economic and health issue due to an increase in gluten-related diseases, as well as an increase in awareness. GF products have poor texture, flavor, and appearance due to the absence of gluten protein, which provides acceptable viscoelastic properties (Gao et al., 2018).

The rheological properties of the dough are dependent both on the quantity and quality of gluten proteins. In addition to retaining fermentation gas, the gluten network can influence the stability of gas cells during expansion, contribute to the characteristics of a soft (spongy) and elastic crumb, and also influence the appearance of wheat-based bread and baked goods (Wrigley, Békés, and Bushuk, 2006). A gluten's extensibility is determined by the gliadin fraction, while its elasticity is determined by glutenin. Gluten exhibits these physical properties that are responsible for the unique viscoelastic characteristics of wheat dough and the quality of wheat-based bread and baked products (Don et al., 2005). As well, they determine the loaf volume and crumb structure of the resulting bread by influencing the gas retention properties of the fermenting dough. Since gluten is absent in gluten-free formulations, dough produced from them does not possess the cohesive and elastic properties obtained from wheat flour. Usually, rice flour is used in the preparation of gluten-free bread due to its colorlessness, nutritional characteristics, bland taste,

***Corresponding author:**
Vikendra Dabash, Tomas Bata University in Zlín, Faculty of Technology, Department of Food Technology, nám. T.G. Masaryka 5555, 76001, Zlin, Czech Republic, **E-mail:** dabash@utb.cz

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and low allergenic properties. The application of enzymes in the production of several GF products is currently an interesting strategy since enzymes can modify protein function, encourage cross-linking of proteins, etc. They can modulate the properties of dough, improve handling and enhance shelf-life.

Eventually, the final product will be consumed (Padalino et al., 2016; Renzetti and Rosell, 2016).

Cereals such as rice, corn, sorghum, and buckwheat, which are free of gluten, are the most recommended gluten-free foods for celiac patients. The quality of the product was significantly affected by corn, buckwheat, and sorghum, except for rice at a concentration of 10-20% (Burešová et al., 2016). Rice is an ideal food for individuals with celiac disease due to its low sodium content and easy digestion of carbohydrates (Corrao et al., 2001). Despite the numerous advantages of rice flour, the absence of gluten proteins makes it nearly impossible to produce a yeast-leavened product such as bread due to the lack of a network capable of storing the carbon dioxide produced by the fermentation process. High-quality GF cereal-based products, such as bread, pasta, cakes, and biscuits, pose a significant challenge. GF products have come under more intense development in recent years. have increased dramatically. Adding functional ingredients and additives such as enzymes to the GF is the simplest way to do it. Researchers have suggested that products mimic the functionality of gluten in order to improve the structure of final products (García et al. 2019; Saeidi and Nasehi 2018).

In the bread-making process, the absence of gluten presents technical difficulties (Diaz and Jouppila 2013). The extensibility and stretchability of gluten-free doughs are less than those of wheat dough. Furthermore, gluten-free dough's viscosity is not optimal during baking. It has been demonstrated that different approaches can be used to overcome this problem, that xanthan gum and carboxymethylcellulose (CMC) can be used as gluten substitutes for preparing bread without gluten (Bonet et al., 2006; Lazaridou et al., 2007; Nishita et al., 1976). Recently, enzymes have also been successfully used in several gluten-free food systems due to their unique ability to modify protein functionality and to facilitate protein cross-linking. Furthermore, enzymes are becoming more popular as consumers become more aware of the use of chemicals in food (Mohammadi, 2021).

Recently, several studies have sought to examine alternative ingredients, including the use of pseudo-cereals (Alvarez-Jubete, Arendt, and Gallagher 2010), dairy products, fibers, and proteins that stimulate the gluten function for improving the rheological characteristics of dough (Ngemakwe et al., 2015). More recently, novel processing techniques, such as enzyme treatment, have been used to enhance the quality and rheological properties of GF dough (Mezaize et al., 2010). Adding α-amylase to dough reduces bread aging by altering the structure of starch. The anti-staling effect of branched-chain products is due to the decrease or interference in the crystallization of amylopectin (Ngemakwe et al., 2015). During the baking process, enzyme activity is an essential factor. Therefore, it is imperative that the enzyme activity of the flour be standardized. With the addition of the enzyme, the α-amylase activity is no longer determined only by the properties of the enzyme, but also by the properties of starch, and it becomes crucial to evaluate how starch structure influences the enzyme activity. Additionally, α-amylase increases the shelf life and softness retention of bread through its anti-staling properties (Błaszczak et al. 2004). The concentration of amylase must be carefully monitored as even small amounts of excess amylase can lead to the production of branched dextrins, which results in gumminess in the bread (Reshu et al., 2017). Specifically, pullulanase is utilized in conjunction with amylase to avoid the gumminess that results from treatment with amylase (Błaszczak et al., 2004).

MATERIALS AND METHODS

Materials

Different types of rice flour (fine flour, semi-coarse, red, white sticky rice, black, red, and extra-fine, manufactured by Flour Spools s.r.o., Brno, Czech Republic ADVENI, while 0.5S flour produced by Extrudo a.s. Týn nad Vltavou, Czech Republic were involved in this study. The composition of the flours was 100% ground rice grains. The fine flour, semi-coarse, white sticky, black, extra-fine, and 0.5S flours were from *Oryza sativa,* whereas the red flour was from *Oryza longistaminata.* The fungal enzyme (α-amylase) originated from *Aspergillus orygal var* was provided by DANISCO Edwin Rahrs Vej DK. Its optimum temperature is 35 to 70 ^{0}C and pH 5-7.5. Dry active yeast, sugar, and salt were obtained from the local supermarket.

Methods

Dough formulation

The formula used to prepare the dough consisted of rice flour (100 g), water (90 g), sucrose (1.86 g), salt (1.50 g), dry yeast (1.80 g), and enzyme α- amylase. A specific amount of α-amylase enzyme was added to the flour in accordance with the falling number, and the enzyme was added until the falling number fell below 62 s. Accordingly, the ingredient amounts were based on 100 g of dry flour. The dried yeast was reactivated in a sugar solution for 10 ± 1 min at (35 ± 1 °C). To prepare the dough, the ingredients were placed in a mixer bowl, and the dough was mixed for 6 minutes using an Eta gratus mixer (Eta a. s. Czech Republic). For the rheological testing, this dough preparation formula was applied to all bread preparations, but dry yeast, sugar, and salt were not added to it.

Moisture content determination

Weighing the flour sample (5 g) and placing it in the moisture meter provided an indication of its moisture content. The sample was heated in the moisture meter to 130°C (temperature heating). In order to determine the moisture content of the flour, it was heated in a moisture meter (OHAUS, MB120). Losses in weight were used to calculate moisture content. The moisture content was expressed as a percentage (%) as shown in Table 1.

Hagberg Falling number determination

It was determined that the amount of flour sample taken was dependent on the moisture content of the flour. The test was conducted using the Perten Hagberg instrument. Each sample was tested twice. Table 1 summarizes the results observed.

Uniaxial elongation test (large deformation)

Textural tests were conducted using a TA XT plus (Stable Micro System Ltd., UK) equipped with a SMS/Kieffer dough and gluten extensibility rig. The sample was stretched until it fractured by hook during the testing process. This test was performed at a speed of 3.00 mm/s with a trigger force of 5 g. The force required to stretch the dough sample as well as the displacement of the hook were recorded as a function of time (Burešová et al. 2014).

Dynamic oscillation measurements

HAAKE RheoStress 1 (Thermo Scientific, Czech Republic) was used for rheological measurements. As described in section 2.2.1, dough samples were prepared manually, excluding dry yeast, sugar, and salt. The elastic modulus G', viscous modulus G'' and η' were determined. Each test was conducted on at least two dough samples (Burešová et al. 2016).

Table 1: A description of the moisture content (%) and falling number (s) in rice flour

Description of rice flour	Moisture content (%)	α-Amylase addition (g/100g)	Control flour Falling Number (s)	After α-amylase addition falling number (s)
Fine	12.40	2	447	62
0.5S	12.60	2	403	62
Semi-coarse	12.90	1.5	467	62
Extra fine	8.69	3	482	62
Red	14.0	1	444	62
Black	13.79	2	>9hrs	62
White sticky	11.84	0.1	494	62

Scanning electron microscopy test

Scanning electron microscopy (VEGA II LMU VG3720771CZ) was employed to examine the microstructures of the dough samples. A gold coating was sputtered over the samples, after which they were observed under an accelerating voltage of 20 kV. A representative photograph was taken at a magnification of 5000 x.

Bread-making and analysis

Bread-making process

Each type of rice flour was made into bread using the same dough and bread-making procedure (Burešová et al. 2016).

Loaf specific volume

Loves were weighted and loaf specific volume was determined in triplicate by using the rape-seed size plastic granulates. Loaf specific volume (mL/g) and baking loss (%) were calculated by given equations.

$$\text{Specific volume (mL/g)} = \frac{Loaf\ volume}{loaf\ weight} \qquad (1)$$

$$\text{Baking loss \%} = \frac{doughweigh - breadweight}{doughweigt} \cdot 100 \qquad (2)$$

Crumb texture parameters and analysis

The textural properties of bread crumb were measured using texture profile analysis (TPA) on texture analyzer TA. XT plus (Stable Micro System Ltd.UK). (Burešová et al., 2014).

Statistical analysis

Using analysis of variance (ANOVA), the TPA for bread and dough results was analyzed statistically. Differences were determined by using the LSD test. Differences were tested at a significance level of $p < 0.05$. The analysis was conducted with the help of Statistica software (Burešová et al. 2014). Small deformation tests (comparing control and enzyme-added dough samples) were conducted using SPSS 20 software. The difference in means was tested at the level of $p < 0.05$.

RESULTS

Behavior of dough during uniaxial elongation (Long-deformation)

A significant impact of the addition of α-amylase enzyme to doughs was only observed with doughs prepared from extra-fine and 0.5S flours. Following the addition of α-amylase to the extra-fine and 0.5S doughs, respectively, the resistance to extension (R) decreased from 0.26 N to 0.11 N and 0.16 N to 0.09 N. The most resistant dough was white sticky. In the presence of the enzyme, the dough was less likely to elongate. The elongation could not be measured, as per Table 2. Physical examination of the extra-fine flour and 0.5S flour indicated that they were very fine indeed. Due to the flour particle size, damaged starch molecules, and water absorption capacity of the dough, the R for the 0.5S dough was lower than that for the extra-fine flour. This is to be expected. With the exception of the black and 0.5S doughs, there was no significant difference in extensibility. We found that by adding enzymes to the black (6 mm), and 0.5S (9 mm), doughs, the L values significantly decreased (by 3 mm and 3 mm, respectively). Other doughs did not exhibit a significant effect of enzyme addition. By adding enzyme to the control dough samples, the area under the curve (A) was reduced; the A values for black dough (0.26 N.mm), 0.5S (1.00 N.mm), and extra-fine dough (0.39 N.mm) were decreased respectively by adding enzyme to 0.13, 0.11, and 0.39 N.mm. Using the results in Table 2, the lowest R (0.26 N.mm), highest A (1 N.mm) and highest L (9 mm) values were recorded in the extra. The impact of α-amylase gel addition was relatively weak during the uniaxial deformation test since only mechanically damaged starch particles were susceptible to attack by enzymes at room temperature. Finer flours were found to be most affected by this effect as the percentage of damaged starch granules in finer flours was higher. A protein called α -amylase hydrolyzes the bonds in large, α-linked polysaccharides like starch to produce glucose and maltose. Similarly, in the results, it was observed that the dough area and extensibility of black rice flour were significantly altered after enzyme treatment. Starch granules are insoluble before being heated in water and will absorb only a limited amount of water.

On both the semi-coarse and white sticky rice dough with enzyme addition (control and enzymes added), the uniaxial test could not be performed.

Table 2: An analysis of dough behavior because of uniaxial elongation.

Flour (Rice)	α-Amylase addition (%)	R (N) (Means)	Area (N. mm) (Means)	L (mm) (Means)
Fine flour	0	0.09 ± 0.01^{a}	0.24 ± 0.05^{cd}	6.00 ± 0.60^{ef}
Fine flour	2	0.07 ± 0.03^{a}	0.13 ± 0.07^{bc}	5.00 ± 2.00^{ef}
0.5 S	0	0.16 ± 0.21^{b}	0.60 ± 0.07^{g}	9.0 ± 0.40^{g}
0.5 S	2	0.09 ± 0.01^{a}	0.11 ± 0.03^{ab}	3.00 ± 1.00^{bc}
Semi-coarse	0	N/P	N/P	N/P
Semi-coarse	1.5	N/P	N/P	N/P
Extra fine	0	0.26 ± 0.02^{c}	1.00 ± 0.08^{i}	9.00 ± 0.80^{g}
Extra fine	3	0.11 ± 0.06^{a}	0.39 ± 0.03^{f}	8.00 ± 1.00^{g}
Red	0	0.08 ± 0.01^{a}	0.08 ± 0.01^{ab}	3.00 ± 0.50^{bc}
Red	1	0.10 ± 0.01^{a}	0.05 ± 0.01^{ab}	1.00 ± 0.50^{ab}
Black	0	0.09 ± 0.01^{a}	0.26 ± 0.06^{de}	6.00 ± 1.00^{ef}
Black	2	0.08 ± 0.01^{a}	0.13 ± 0.02^{b}	3.00 ± 0.60^{cd}
Sticky white	0	0.41 ± 0.04^{d}	0.88 ± 0.10^{h}	5.00 ± 1.00^{de}
Sticky white	0.1	N/P	N/P	N/P

R: resistance to extension of dough; A: extension area; L: extensibility of dough. The mean values indicated by the letters in the column differ significantly ($p<0.05$). N/P - No test was performed

Dynamic oscillation test of different types of rice flour doughs

It has been determined that the behavior of dough on the heat is demonstrated in the different small deformation properties (G' G'' *and* η') for all different rice flours (used in the study), for both (control and enzyme-added) samples. Table 3 shows the heat-dependent variations in the storage modulus (G') and loss modulus (G'') for all control dough samples and enzyme-added dough samples respectively. As a result of the addition of α-amylase enzyme in this study, the red and white-sticky doughs' viscous modulus (G'') was increased for the enzyme-added dough sample over the control dough sample. The fine, extra-fine, and black dough samples had a reduced viscous modulus after enzyme addition. After enzyme addition, elastic modulus (G') was decreased in all samples with the exception of red and white-sticky doughs as shown in Table 3. A significant increase in the viscosity of the 0.5S, red, and white-sticky dough samples (η') was observed when enzyme was added as compared to the control dough sample. Despite enzyme addition, all the measured samples' elastic modulus was less than their viscous modulus except for the 0.5S dough sample. In the presence of only 0.5S with enzyme, the viscoelastic modulus of dough samples was significantly higher than the viscous modulus ($G'' > G'$). The oscillation test was not conducted on both semi-coarse dough samples (control and enzyme-treated). It appeared that the flour particles (as seen physically) were larger in size than others in the dough. As a result, the dough's capability of absorbing water was relatively low, and less damaged starch had been included in the flour compared to other flours tested. Prior to heating, the α-amylase enzyme attacks only the damaged starch granules. Except for semi-coarse dough samples, all other tested dough samples showed significant changes in all rheological parameters after enzyme addition in comparison to the control. Using black rice as a control, the elastic modulus (G') was higher than the enzyme-added samples with means of 14000 Pa. Similarly, as shown in Table 3, the viscous modulus (G'') of control dough samples was higher than that of enzyme-added samples.

This indicates that the control dough is stiffer in texture than the enzyme-added dough sample. The results from this study suggest that endogenous α-amylase significantly reduced the viscoelasticity of black rice flour dough. A decrease in loss modulus corresponds to a decrease in the hardness of the dough. It can be seen from Table 3 and Figs 1 & 2 that $G' > G''$ represents the elastic behavior of the dough in both samples (control and with enzyme addition). Viscosity (η') in control samples recorded a very high value of 8600 Pas when compared with enzyme added dough sample with 2300 Pas, as shown in (Fig 3, and Table 3). Possibly because starch is hydrolyzed, and α-amylase reacts with the damaged starch in the flour dough.

Table 3: The effect of α Amylase enzyme on the rheological properties of rice doughs

Rice Flour Dough	Elastic modulus *G’* Pa		Viscous Modulus *G”* Pa		Viscosity *η’* Pas		*p* value
			Mean				
	Control	Enzyme	Control	Enzyme	Control	Enzyme	0.000**
Fine	110000	22000	15000	4000	8000	4000	0.000**
Extra fine	140000	32000	18000	5000	23000	5000	0.000**
0.5S	141000	29000	21000	134000	23000	214000	0.000**
Black	53000	14000	8000	2900	8600	2300	0.000**
Red	300000	330000	53000	58000	48000	53000	0.000**
White sticky	162000	257000	41000	54000	27000	42000	0.000**
Semi-coarse	N/P	N/P	N/P	N/P	N/P	N/P	N/P

G’: Elastic modulus; *G”*: Viscous modulus; ▯’: Viscosity, the mean values differ significantly ($p < 0.05$) **. *N/P* -Test was not performed

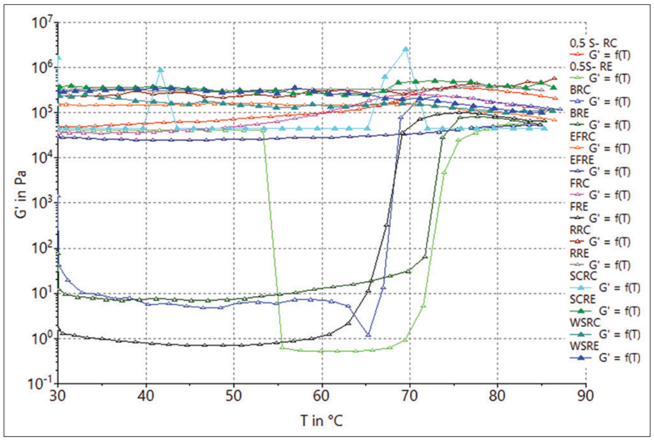

Fig 1. The change in the elastic modulus (*G’*) after α-amylase enzyme addition in the rice flours at 30 - 90 °C temperature.
Where: RC- Rice control, RE- Rice with enzyme addition BR-Black rice; EF- extra fine; FR- fine rice; RR- red rice; SC- semi-coarse; WS-white-sticky.

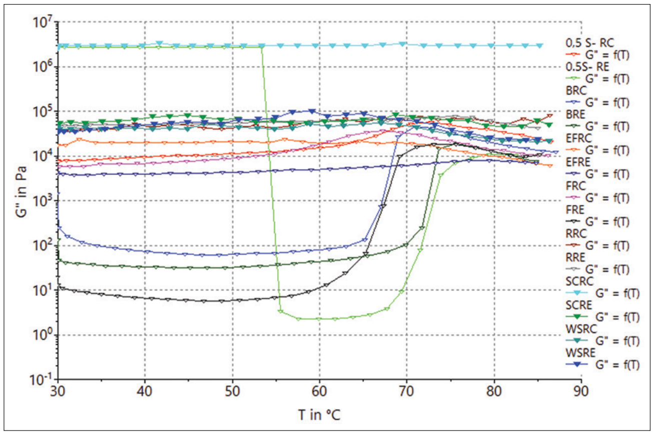

Fig 2. The change in the Viscous modulus (*G”*) after α-amylase enzyme addition in the rice flours at 30 - 90 °C temperature.

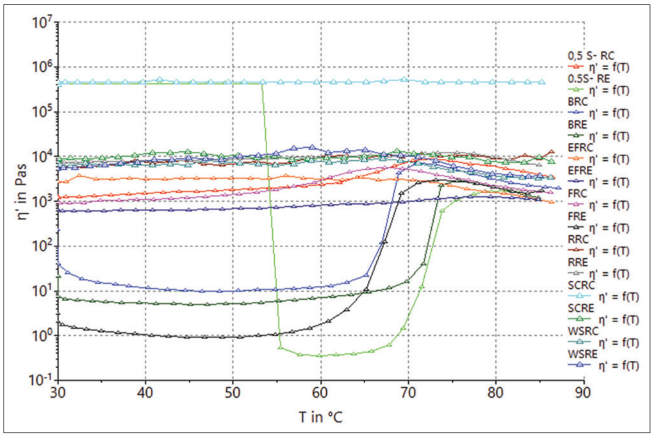

Fig 3. The change in the Viscosity (η’) after α-amylase enzyme addition in the rice flours at 30 - 90 °C temperature.

The storage modulus, loss modulus, and viscosity of dough samples containing red rice flour and enzymes were increased by 330000 Pa, 58000 Pa, and 53000 Pas, respectively. The values of all three parameters (*G’, G”,* and *η’*) were increased following the addition of the α-amylase enzyme. The following table indicates that *G’* > *G”* indicates a similar elastic behavior for both samples (control and with enzymes added). When enzymes were added to fine flour dough, all three parameters showed significant reductions in values. A reduction of 22000 Pa, 4000 Pa, and 4000 Pas were made to the *G’* 110000 Pa, *G”* 15000 Pa, and η*’* 8000 Pas, respectively. After the addition of enzyme, *G’* in the control sample of 0.5S rice dough decreased. Similarly, *G”* and η*’* values were increased in the dough sample after enzyme addition. It was shown that the G’ value of 0.5S rice dough without enzyme addition (control) was significantly decreased by the α- amylase enzyme added 0.5S dough sample. Moreover, *G”* and η*’* values of 0.5S rice dough without enzyme addition (control) were significantly increased when α-amylase enzyme was added to 0.5S dough sample. Table 3 and Figs 1 & 2 demonstrate that the G’ > G” indicates that the control dough sample exhibits elasticity.

Compared to the extra fine rice flour dough without enzyme addition (control) (140000 Pa, 18000 Pa, and 23000 Pas), the three rheological parameters (*G, G”,* and η*’*) were significantly decreased by the extra fine rice flour dough containing the α -amylase enzyme (32000 Pa, 5000 Pa, and 5000 Pas, respectively). By adding the enzyme α -amylase

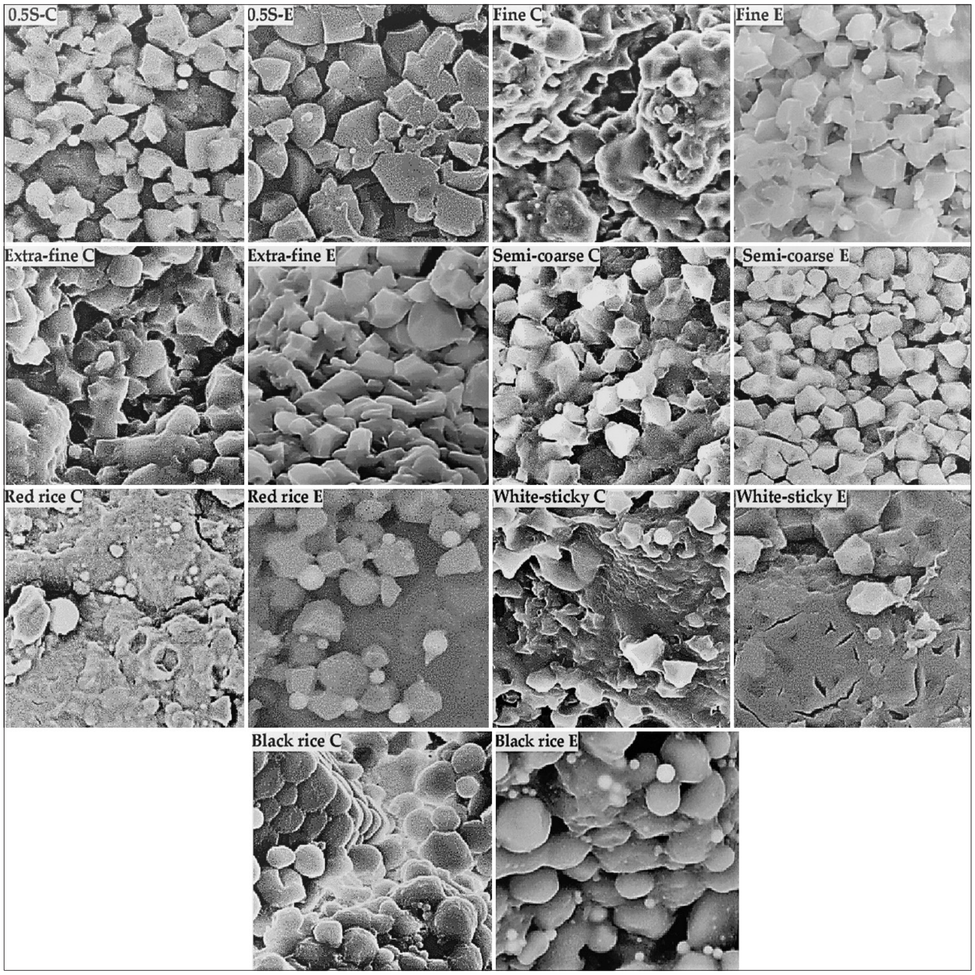

Fig 4. SEM images of the doughs (control and with enzyme addition) after kneading. C- Control, E- with the enzyme.

to white sticky dough samples, the elastic modulus, and viscosity were decreased. As shown in Fig 1 and 2 as well as Table 3, *G' > G'* showed the elastic behavior for both samples of white sticky rice flour dough (with and without enzymes). In comparison with control dough sample with a viscosity of 27000 Pas, enzyme added sample with a viscosity of 42000 Pas has a high value. As the viscosity increased, the consistency of the dough increased. When the dough samples were semi-coarse (Control and enzyme added), the curves for G', G'', and *η'* did not have the proper shape. It was not able to calculate the graph curve and the values of the above parameters. It is possible that this curve shape is due to the particle size of the flour or the 90% water amount was insufficient to produce a dough texture.

Scanning electron microscopy

In the present study, a scanning electron microscope (SEM) was used to characterize the sample microstructure after the kneading process in order to assess the effect of the 'amylases on the microstructural properties of different types of rice dough samples. Fig 4 depicts representative SEM micrographs of doughs with enzymes added and without enzymes added. As compared to the control samples, enzyme-treated samples (extra-fine, fine, 0.5S, and red) had a significantly different structure; the control samples consisted of less complexed starch molecules, resulting in a dense and compact structure. Consequently, the specific volume of the bread made with controlled rice doughs decreases In Fig 4, the swollen damaged starch granules are completely different from those found in the control dough samples (fine, extra fine, and 0.5S).

Baking tests

Moreover, the addition of the α-amylase enzyme had no significant effect on baking losses, as shown in Table 4. Accordingly, the 0.5S and extra-fine control samples yielded the largest and smallest specific volumes (1.95 and 1.16 mL/g), respectively.

Despite the addition of enzymes, the highest and lowest specific volumes in the white-sticky bread and red rice samples were found after enzyme addition, respectively. Due to the addition of amylase, the specific volume of the loaf was generally increased, although the effect was significant only

Table 4: Baking loss and specific volume of bread loaves

Flour (Rice)	α-amylase addition (%)	Loaf-specific volume (mL/g) mean	Baking loss (%)
Fine flour	0	1.77 ± 0.03^{bc}	0.19 ± 0.01^{ab}
Fine flour	2	1.89 ± 0.01^{bc}	0.19 ± 0.01^{ab}
0.5 S	0	1.95 ± 0.10^{c}	0.23 ± 0.01^{ab}
0.5 S	2	1.80 ± 0.04^{bc}	0.23 ± 0.02^{ab}
Semi-coarse	0	1.54 ± 0.03^{ab}	0.20 ± 0.02^{ab}
Semi-coarse	1.5	1.68 ± 0.03^{bc}	0.21 ± 0.02^{ab}
Extra fine	0	1.16 ± 0.03^{a}	0.18 ± 0.02^{ab}
Extra fine	3	1.85 ± 0.05^{bc}	0.18 ± 0.01^{ab}
Red	0	1.86 ± 0.06^{bc}	0.20 ± 0.03^{ab}
Red	1	1.97 ± 0.05^{c}	0.20 ± 0.02^{ab}
Black	0	1.79 ± 0.04^{bc}	0.16 ± 0.01^{ab}
Black	2	1.95 ± 0.04^{c}	0.17 ± 0.02^{ab}
White-sticky	0	1.69 ± 0.04^{bc}	0.18 ± 0.02^{b}
White–sticky	0.1	1.69 ± 0.04^{bc}	0.18 ± 0.02^{b}

Mean values followed by the same lower case letter in the same row are not significantly different ($P < 0.05$).

in bread prepared from extra-fine rice flour. Nevertheless, the specific volume of all breads except for extra-fine bread did not change significantly. Table 4 shows that the specific volume of the extra-fine control bread (1.16 mL/g) has been increased by the addition of the α-amylase enzyme (1.85 mL/g) as shown. Table 4 shows that enzyme addition resulted in significant increases in a specific volume of extra-fine bread compared to the control dough bread samples whereas no significant differences were found for other samples (fine flour, red, black, white sticky, and semi-coarse).

Texture profile analysis of bread

An increase in hardness was observed after enzymes were added to fine flour, semi-coarse, and extra-fine breadcrumbs. Crumb hardness was reduced by 2.48, 34.13, 21.35, and 5.46 N in the control fine flour (127.21 N), semi-coarse (26.69 N), and extra-fine (27.20 N) samples by addition of α-amylase. Except for the fine flour, extra-fine, and 0.5S flour, no other bread changed significantly in springiness. Adding enzymes to the control bread significantly reduced its springiness (62.86%) compared to the control bread with 0.5 S. In contrast, enzyme addition resulted in a significant increase (98.26%) in the springiness of the extra-fine control bread (73.73%). The bread crumb samples did not differ significantly in any other way, except for the fine flour and extra-fine breadcrumbs. Specifically, the

These values represent the averages of three replicates with six standard errors. The differences between mean values followed by the same lower-case letter in the same row are not significant (P<0.05). N/P-tests were not performed. Semi-C is semi-coarse, and White-S is white sticky.

Chewiness of the fine flour control bread crumb (613.17) and the extra-fine control bread crumb (154.94) decreased by adding the enzyme α-amylase (148.44 and 40.16, respectively) as shown in Table 5.

Compared to the control loaf, the extra fine and semi-coarse breads with enzyme addition had significantly greater crumb resilience. With enzyme addition, extra-fine (40.13%) and semi-coarse (38.68%) breadcrumbs became more resilient (50.30% and 52.01%, respectively). As a result, the yeast may have fermented the damaged starch in the dough by breaking it down into smaller dextrins. However, the resilience (37.04%) of the fine flour enzyme-added crumb samples was less than the (44.48%) control crumb samples, since the enzyme had little effect on the outer amylopectin chains, amylopectin, and recrystallization, and, therefore, did not impede water immobilization. Furthermore, a significant difference was found in the durability of the fine flour bread crumb. As shown in Table 5, the resilience of the enzyme-added fine flour bread crumb (60.15%) was lower than the resilience of the control fine flour bread crumb (73.97%). It is not realistic to generalize regarding the production of gluten-free bread, since a complex system is involved, so the addition of amylase enzyme to rice dough will not necessarily result in good-quality bread.

DISCUSSION

Using rheological analyses, we found that 0.5S and extra fine flour dough had significantly different levels of resistance to extension and area. Despite the addition of enzymes, the extensibility results of extra fine flour did not significantly change, but the extensibility of black rice and 0.5S dough did significantly change after enzyme addition. According to (Shukla et al., 2015), the attack by α -amylase occurs only during mechanically damaged starch granule tests and other tests performed on dough that occur below the gelatinization temperature. It could be related to its ability to catalyze the oxidation of glucose into gluconic acid and hydrogen peroxide, the process of which induces the gelation of water-soluble pentosans within rice flour (Bonet et al., 2006). In this instance, results obtained from baking the dough after enzyme addition found that the specific loaf volume differed only significantly in extra fine flour dough breads. The specific volume was increased by α-amylase (Gujral, Haros, and Rosell, 2003). As a result, native starch is more soluble than cross-linked starch formed during baking, giving a shorter texture as it reduces breaking and solubilization, and the gel strength of baked amylose is decreased due to the increased degradation of cross-links (Lazaridou et al., 2007). The results of testing showed that extra fine flour dough (control and when enzyme was added) was more elastic than other rice flour doughs. The control dough may have a more rigid structure than the enzyme-infused dough. Consequently, the dough added with an enzyme became softer than the control dough.

Table 5: The bread Crumb texture Characteristics of rice flour bread.

Flour (Rice)	α-amyl(%)	Hardness N (Mean)	Springiness (%) (Mean)	Chewiness (Mean)	Resilience (%) (Mean)	Cohesiveness (%) (Mean)
Fine	0	127.21±5.00^{h}	65.08±3.00^{ab}	613.17±229.00^{d}	44.48±9.00^{cdefg}	73.97±25.00^{cdef}
Fine	2	34.13±0.90^{i}	72.28±2.00^{abcd}	148.44±6.00^{c}	37.04±1.00^{ab}	60.15±0.70^{a}
0.5S	0	4.80±1.00^{ab}	76.74±3.00^{bcd}	27.25±5.00^{a}	45.44±0.80^{defgh}	74.40±2.00^{cdef}
0.5S	2	3.70±1.00^{a}	62.86±15.00^{a}	17.91±10.00^{a}	45.66±10.00^{def}	72.44±5.00^{cdef}
Semi-C	0	26.69±3.00^{g}	75.50±4.00^{abcd}	143.10±21.00^{c}	47.27±1.00^{efgh}	70.92±1.00^{bcdef}
Semi-C	1.5	21.35±7.00^{f}	70.24±18.00^{abc}	105.21±42.00^{bc}	38.68±8.00^{abc}	68.49±8.00^{bcd}
Extra fine	0	27.20±7.00^{g}	73.73±3.00^{abcd}	154.94±43.00^{c}	52.01±2.00^{h}	76.98±1.00^{ef}
Extra fine	3	5.46±0.40^{abc}	98.26±42.00^{e}	40.16±18.00^{a}	48.57±3.00^{fgh}	74.63±2.00^{cdef}
Red	0	8.21±1.00^{cde}	73.02±1.00^{abcd}	38.41±6.00^{a}	40.96±2.00^{bcde}	64.08±0.80^{ab}
Red	1	9.19±0.60^{de}	68.29±2.00^{abc}	42.56±2.00^{a}	41.85±0.90^{bcd}	67.87±0.70^{bc}
Black	0	10.79±2.00^{e}	81.38±2.00^{cd}	60.35±12.00^{ab}	44.85±1.00^{defg}	69.01±1.00^{bcdef}
Black	2	5.13±0.4^{abc}	86.52±2de	31.75±2^{a}	47.78±2^{fgh}	71.56±0.50^{cdef}
White-S	0	5.14±0.70^{abc}	75.50±2.00^{abcd}	29.20±4.00^{a}	34.64±0.90^{a}	75.31±2.0^{def}
White-S	0.1	N/P	N/P	N/P	N/P	N/P

There are several enzymes that are common in GF breads, such as α-amylase and thermolysin, amyloglucosidase, maltogenic amylase, cyclodextrin, among these enzymes are glycosyltransferase (CGTase), glycogenase (TGase), glucose oxidase (GOO), laccase, and lipase, and protease (Matos and Rosell 2015). Enzymes help to improve the quality, shelf life, and handling of GF breads by increasing the functionality of proteins and enhancing shelf life. In a previous study, α-amylase and amyloglucosidase added to bread made with quinoa resulted in a 23% increase in volume and a significantly improved firmness (Elgeti et al., 2014). In fact, α-amylase hydrolyzes α-(1–4) bonds of starch and low molecular weight α-dextrins are produced, which leads to a decrease in dough resistance during fermentation (Kawamura-konishi et al., 2013).

Following the addition of enzymes, the viscosity of red rice, and white sticky rice flour doughs increased, whereas the black, fine, 0.5 S, extra fine, and semi-coarse rice flour doughs also became viscous after the enzyme addition. Therefore, the small deformation results are correlated with the uniaxial elongation results. Rice flour must be chosen carefully since different types can have a significant impact on the final quality of products. Following enzyme addition, after dynamic oscillation test, G’<G” was observed in both the 0.5S and fine rice dough. Essentially, the dough was more viscous than the control dough. -Amylase releases water from damaged starches, which results in a decrease in the storage modulus (Dog, 2002). The reduction in the storage modulus also mimics an increase in water absorption, as shown by (P. C. Dreese, J. M. Faubion, 1988). There were significant differences in TPA results for bread crumb fine flour dough as well as extra fine flour dough. The obtained results may indicate a superior quality of bread made from extra-fine rice, which is consistent with the report of (Gujral et al., 2003) who found that the addition of amylase enhances the volume and crumb of rice bread. Generally, the use of α -amylase is based on its softening effect on the crumb, as well as the improvement in flavor and volume. Consequently, it can be expected that α -amylase will have an effect on baking. By the time the granules reach the gelatinization temperature, the structure is starting to collapse, and starch is being hydrolyzed by α-amylase. We studied the rheological properties of various types of rice flour dough. The rheological parameters (*G’, G”’ and* η’) of the black rice flour, fine rice flour, and extra fine rice flour doughs all decreased with the addition of enzyme. Conversely, the rheological parameters of the red rice and white sticky rice flour dough samples increased with the addition of enzyme. The elastic modulus, viscous modulus, and viscosity values were likewise increased after enzyme addition in the remaining semi-coarse and 0.5S rice dough samples. Additionally, only extra fine, 0.5 S, and black flour doughs experienced a significant change in parameters after the addition of enzymes. There is an assumption that the differences in rheological parameters after enzyme addition may be caused by varying amounts of damaged starch in the rice flour because alpha amylase enzymes attack damaged starch only (James BeMiller and Roy Whistler, 2009). Microscopical examination of rice doughs revealed a fine degradation of starch, which supports the connection between changes in dough rheology and molecular interactions. In Fig 4, the starch granule microstructures (extra fine, 0.5S, fine, and semi-coarse) were observed to be more visible after enzyme addition than in the control dough samples. It has been suggested that dextrins may interfere with the interactions between swollen starch granules and proteins (Gray and Bemiller, 2003). The same microstructure was also observed in the rice starch gel by (Zhang et al., 2021).

CONCLUSIONS

According to the results of this study, the use of alpha-amylase enzyme in GF rice flour can enhance the texture of both bread and dough, as well as rheological properties. The results show that using the alpha-amylase enzyme can increase the quality of the final GF rice flour product. Based on the results obtained, rice flours combined with alpha-amylase may be incorporated into GF product formulations. Therefore, further research is required to improve textural and rheological quality and to increase the quality of GF products. According to the results obtained, enzyme addition could significantly improve the quality of the final product (bread) obtained from extra-fine and fine flour dough for bread. A study of the effect of α-amylase on dough and bread rheological properties did not find a statistically significant difference. An analysis of the dough texture profile revealed that the extra fine, 0.5S and black rice flour doughs had significant differences. In microstructure results the control dough samples, the starch granule microstructures were complex and not easily visible. it can be assumed that the α -amylase enzyme can be utilized to develop fine rice flour products. It is possible to improve the quality of rice flour dough breads by adding alpha-amylase enzyme.

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Author contributions

Conceptualization, V.D., and I.B.; methodology, V.D., validation, I.B.; formal analysis, V.D.; data curation, I.B., and V.D.; writing—original draft preparation, V.D.; writing-review and editing, I.B and V.D.; visualization, I.B.

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